

## Effect of Irrigation Water and Spraying with Proline Acid on Growth and Yield Components of Wheat *Triticum Aestivum* L

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In this greenhouse experiment conducted at one of Baghdad's nurseries during the 2018-2019 growing season, we investigated the impact of varying concentrations of well water (ranging from 0 to 4.3 dS/liter) and proline acid (at concentrations of 0, 10, 20, and 30 ppm) on key indicators of vegetative growth and yield components in the Tammuz variety of wheat plants. Employing a completely randomized design with three replicates, we observed a significant decrease in vegetative growth and yield components as irrigation water levels increased from 0 to 4.3 decimen/liter. Interestingly, foliar spraying with proline acid exhibited a noteworthy increase in both growth indicators and yield components. Furthermore, our results highlighted the mitigating effects of proline acid application on the negative impacts associated with high concentrations of irrigation water. This underscores the potential of proline acid as a beneficial intervention to counteract adverse effects on various growth parameters and yield components in wheat plants under high irrigation concentrations.

**Keywords:** Wheat, well water, proline, spraying, yield components, greenhouse experiment, well water concentrations, proline acid concentrations, growth indicators.

### INTRODUCTION

Wheat is one of the most important cereal crops and belongs to the Gramineae family. It is the first cereal crop in Iraq and the world in terms of nutritional, industrial and economic importance, cultivated areas and general production. In Iraq, the area planted with the wheat crop was estimated at 7376 thousand dunums for the winter season 2013. The cultivated area in the irrigated lands was estimated at 5062.8 thousand dunums, while the lands planted in the fertile lands were estimated 23133.4 thousand acres, representing 31.4% of the total area (Hussein, and Khursheed, 2014). This large area is required to provide large quantities of water in order to sustain agriculture and production, which is offset by the scarcity of rainfall and water resources in the Tigris and Euphrates rivers (Parveen *et al.*, 2021). Salinity is also one of the most important factors of abiotic stress that determine the growth and productivity of plants in general and wheat in particular. There is evidence of the effects of salts on photosynthetic enzymes, chlorophyll, carotenoids, the ability to photosynthesis, changes in water stress and leaf swelling pressure, as cumulative effects attributed to salt stress were recorded, as well as some soils and other environmental

factors that have an impact on plant growth under saline conditions. On the other hand, not all data give an indication of a positive association between osmolyte pool and stress adaptation.

(Kateb, 1988).

Increasing the amounts of salt in the soil has harmful effects on the growth and detection of plants represented by the following: seed germination, seed growth, vegetative growth, flowering and fruit formation, and consequently the poor quality of the product. The plants were classified into: salinity sensitive and tolerant of salinity, according to their ability to grow in high concentrations in the saline medium. The salinity-sensitive type cannot tolerate salt stress, and high concentrations of salt reduce the osmotic stress of the soil solution and cause water stress for plants, as well as because of the toxicity of sharp ions such as Na<sup>+</sup>, as it is not easily segregated within the gaps, and finally the interaction between salt and nutrients causes a nutritional imbalance. There is another classification of salinity by (Ghassemi *et al.*, 1995), where salinity is classified into: primary and secondary.

Primary salinity: results from the accumulation of salts for long periods according to natural processes, where the process of weathering of rocks containing various dissolved

salts takes place at first, diagnosed mainly to calcium, sodium and magnesium chlorides. Secondary salinity: the accumulation of salt carried by wind or rain (Ibrahim and Samiullah, 2010).

Salt stress negatively affects plant growth, affecting cell division and expansion, thus affecting the stages of DNA building, as well as affecting the opening and closing of stomata, thus affecting the photosynthesis process by affecting the decline of light systems, inhibiting the activity of Rubisco enzymes and decreasing the content of photosynthetic pigments, as well as the oxidative phosphorylation process, hormonal imbalance and protein content were affected, due to the decrease in the polyribosom content and the decrease in nitrate flux due to the decrease in the activity of the enzyme nitrate reductase (Yassin, 2001).

Salt stress has an effect on the phenotypic, physiological and biochemical characteristics of the plant, so the effect is directly on the growth and development of the plant, and thus the effect of salts is osmosis or ionic, so the plants exposed to the influence of environmental stresses have the ability to withstand this effect through a change in their metabolic processes (Munns, 2002). Nevertheless, the tolerance of plants to salinity varies according to plant species (Rabie, 2005). Plants adapt to some harsh environmental conditions such as drought and high salinity by collecting some amino acids, especially the amino acid proline, which has an important role in the osmotic regulation of the cytoplasm in cells as a complementary solute (Bartels, 2005).

Proline acid is an amino acid that has the ability to regulate osmosis inside the cell, specifically between the vacuole and the cytoplasm under conditions of salt stress. The ions accumulate in the vacuole, which leads to the occurrence of osmotic imbalance conditions inside the cell and this leads to the accumulation of proline in the cytoplasm to reach the state of equilibrium. Proline acid has an important role in maintaining cell swelling and maintaining enzymatic activity in the cytoplasm by keeping enzymes from degrading as well as maintaining the membrane structure of cellular organelles (Yassin, 1992).

The proline gathering during osmotic stress also has a role in increasing the ratio of NADPH<sup>+</sup> and NADP that enters the cellular metabolism process, and thus has a role in the process of aerobic respiration in the cell as a source of energy. Therefore, it helps the plant to get rid of the state of stress that it has been exposed to and heal and also helps the plant to return to the natural state, in addition to its role in scavenging oxidative free radicals (Hare, 2004). Oxidative stress is the result of damage caused by exposure to drought, high salinity, radiation, diseases and heat, which lead to the production of oxidative free radicals (ROS) (Reactive Oxygen Species) as a product of the metabolic processes that occur in the plant. Among the most important of these radicals are the superoxide radical and the hydroxyl radical, in addition to the single oxygen radicals, where these radicals negatively affect

the fatty part of the plasma membrane, causing its oxidation, thus affecting the membrane's permeability, and it also has an effect on nucleic acids and building proteins (Gratao, 2008).

In a study conducted by (Tan and Zhao 2008) on wheat plant exposed to osmotic stress, they indicated an increase in the accumulation of oxidative free radicals, thus affecting plant growth, and that the increase in the plant's ability to collect proline acid helped remove the negative effects of these radicals as a scavenger of oxidative free radicals. (Al-Saadi, 2010) indicated in their study on wheat plant growing in a medium which irrigated by well water with different concentrations of irrigation water, the role of irrigation water in reducing plant growth, and thus significant decreases occurred in the average components of the yield and that spraying the vegetative part of the plant with concentrations of proline acid led to reducing damage caused by irrigation water and a noticeable increase in the plant's adaptability to salinity, which led to an increase in all yield components values for wheat plants. Given the importance of wheat as one of the economically important grain crops and because of the lack of rainfall and irrigation water, which led to an increase in drought and an increase in the area of saline agricultural lands in Iraq. The aim of this experiment was to identify the role of proline acid as an osmotic regulator in reducing the negative damage to irrigation with different concentrations from well water on wheat plant growth.

## MATERIALS AND METHODS

The experiment was conducted in the greenhouse of one of Baghdad's nurseries for the 2018 growing season using clay soil, air-dried, crushed, sifted and packed into plastic pots of 7 kg of soil per pot. The experiment was carried out according to a completely randomized design as a factorial experiment (4x4) and with three replications. It included the following factors:

1. Three levels of water (well water) (1.6, 3.2, and 4.3) ds/liter, which were brought from villages that lack good well water and have salty well water.
2. Three concentrations of proline acid (10, 20 and 30) ppm. A main solution of 1 gm of proline acid was prepared and dissolved in a liter of distilled water, then the required concentrations were prepared according to the dilution law as well as the concentration 0 as a control treatment.

As such, the number of experimental units is 48. The potting soil was fertilized using calcium superphosphate fertilizer at a rate of 0.7 gm. Pot<sup>-1</sup>. Also, urea fertilizer was added at a rate of 0.35 gm. Pot<sup>-1</sup> as a first batch. After 45 days of planting, a second batch of urea fertilizer was added at a rate of grams. Pot<sup>-1</sup> 0.35 (Al-Qazzaz, 2010). Wheat seeds of Tammuz variety were sown on 23/11/2018 at a rate of 15 seeds per pot, and irrigated with water to reach 50% of the field capacity.

The seedlings decreased to 10 after 14 days of planting, and the agricultural operations of irrigation and removal of the



bush were followed up until the end of the experiment. When the leaf 3-4 appeared, it was irrigated using well water and sprayed with concentrations of proline acid prepared according to the treatments in the morning on the vegetative part of the plant on 2019/ 1/14. The spraying was evenly carried out until complete wetness. The control treatments were sprayed with distilled water while continuing to irrigate with well water. After 14 days of the first spray, the second spray was carried out with the same concentrations of proline acid, while continuing to be irrigated with well water. Some indicators of vegetative growth have been studied as follows:

1. Plant height: measurement was taken from the point of contact of the plant with the soil surface to the highest point in the plant on 2/5/2019.
2. The total chlorophyll content of the flag leaf was measured using the SPAD device on 8/3/2019 wheat plants were harvested on 19/4/2019 and some components of the yield were calculated:
  - a- Grain yield (gm. Pot<sup>-1</sup>)
  - b- is the grain weight of the sample harvested at 12% moisture (1975, A.O.A.C) b- The number of grains in the spike? The number of grains was calculated in 5 ears of each pot, which were randomly selected after the grains reached maturity.
  - c- Weight of 100 tablets (gm)

One hundred grains of the grain yield of each pot were weighed by the sensitive scale. The obtained data were analyzed statistically according to the design of the experiment according to method (Little, 1978) and the means were compared using the least significant difference at the probability level of 0.05.

## RESULTS AND DISCUSSION

High salinity is one of the most environmental factors that limit plant growth, as high concentrations of sodium ion affect soil tissue and reduce its porosity and aeration, thus reducing its ability to hold water, which affects the plant's ability to absorb water and nutrients from the soil, thus affecting vital metabolism of the plant, which is negatively reflected in the growth and elongation of the plant (Ashraf, 2008). High salinity affects the hormonal balance of the plant, as it reduces the biosynthesis of growth hormones such as cytokinin and gibberellin, which have a role in elongating the stems. Salinity increases the accumulation of some growth inhibitors that upset the balance of internal hormones in plant tissues, such as abscisic acid (Rabie, 2005). Spraying with proline acid led to an increase in plant height, and this is due to the positive role of proline acid in the osmotic reorganization after saline stress conditions, which is considered one of the buffer solutions and preserves the cell from oxidation in addition to being an enzymatic preserver and maintains cellular structures, so it contributes

to increasing plant growth and perpetuate cell elongation, stomata opening, and increase photosynthesis (Hare, 2004). The results in Table (1) indicate a significant decrease in the average plant height by increasing the salinity level of the irrigation water from 0 to 4.3 decimens/liter, with a decrease of 18.48%, and that foliar spraying with proline acid reduced the negative effect of water salinity. From 0 to 30 ppm, there was a significant increase in the average plant height of 14.53%, as the highest average plant height was 44.74 cm at concentration 20 ppm compared with concentration 0 of proline acid with an increase of 16.11% and the effect was not significant between concentrations of 20 and 30 ppm of proline acid in average plant height. As for the interaction effect between the study factors, it was not significant.

**Table 1. Effect of irrigation water concentrations and spraying with proline acid and their interactions on plant height (cm) of wheat.**

Proline (ppm)	Well Water Concentration (ds/liter)				Mean
	0	1.6	3.2	4.3	
0	42.00	40.00	37.60	34.50	38.53
10	44.90	44.30	40.00	38.60	41.95
20	47.30	45.30	45.00	41.35	44.74
30	50.30	43.60	43.80	38.81	44.13
Mean	46.38	44.05	41.10	37.81	

L.S.D. Well water = 1.502; Proline = 1.502; Interaction = N.S.

The decrease in chlorophyll of the flag leaf resulting from weak plant growth in a saline medium is due to a decrease in the content of the elements involved in building the chlorophyll molecule such as nitrogen and magnesium as a result of their lack of absorption. It also inhibits the enzymes responsible for building photosynthetic pigments. The salt environment negatively affects the leaf area and decrease CO<sub>2</sub> concentration in chloroplasts due to stomata closing, thus affecting the process of transpiration and photosynthesis (Yassin, 1992).

The increase in the total chlorophyll content of the flag leaf is due to spraying with proline acid, which has a positive role in stimulating it by building chlorophyll pigments and forming plastid granules, and it has a role in maintaining the enzymatic activity of the plastids, which leads to delaying leaf aging (6). The increase in the level of salinity of irrigation water in the growth medium led to a significant decrease in the average content of total chlorophyll of the flag leaf of wheat plant, as shown in Table (2). In the trait averages with a decrease of 20.15%, and when the concentration of proline acid was raised from 0 to 30 parts per million, there was an increase in the trait averages by an amount of 21.49%, and the highest mean for the trait at the concentration of 20 ppm of proline acid was 38.76 micrograms. cm<sup>2</sup>- with an increase of 21.96% compared with the chlorophyll content at concentration 0 of proline acid, and the difference was not significant between 20 and 30 ppm proline acid



With regard to the interaction effect between the two factors of the study, it was significant, and the highest value of chlorophyll content in the flag leaf was at level 0 for irrigation water with a concentration of 30 ppm of proline acid, and it amounted to 42.40  $\mu\text{g. cm}^{-2}$ .

**Table 2. Effect of concentrations of irrigation water and spraying with proline acid and their interactions on the total chlorophyll content ( $\mu\text{g. cm}^{-2}$ ).**

Proline (ppm)	Well Water Concentration (ds/litter)				Mean
	0	1.6	3.2	4.3	
0	38.00	33.80	29.80	25.50	31.78
10	40.25	37.90	36.15	32.85	36.79
20	40.90	39.20	37.75	37.20	38.76
30	42.40	38.10	37.70	36.25	38.61
Mean	40.64	37.50	35.35	32.45	

L.S.D. Well water =0.452; Prolien = 0.452; Interaction =0.903

The growth of the plant in a medium containing high concentrations of irrigation water as a result of irrigation with well water negatively affected its growth, which affected the components of the yield. The results in Table (3) indicated a significant decrease in the rate of grain yield ( $\text{gm.pot}^{-1}$ ) for wheat plant by 41.32 % when the water salinity level was increased from 0 to 4.3 dSi / liter, and when the concentration of proline acid was raised from 0 to 30 ppm, there was a significant increase in the characteristic rate with an increase of 11.91%. The difference was not significant between the concentrations 20 and 30 ppm proline acid in the characteristic rate. As for the effect of the interaction between the type of irrigation water and proline acid, it had a significant effect, as the highest value of the character at the concentration 0 for the water type and the concentration was 30 ppm proline acid and it reached 0.940 g compared with the lowest value for the character at the concentration of 4.3 dSiemens / liter for the water type and the concentration 0 acid. Proline, which amounted to 0.475 gm with a decrease of 49.46%.

**Table 3. Effect of concentrations of irrigation water and spraying with proline acid and their interactions on grain yield ( $\text{gm.pot}^{-1}$ ).**

Proline (ppm)	Well Water Concentration (ds/litter)				Mean
	0	1.6	3.2	4.3	
0	0.815	0.659	0.535	0.475	0.630
10	0.860	0.750	0.580	0.520	0.665
20	0.880	0.750	0.585	0.530	0.686
30	0.940	0.770	0.580	0.530	0.705
Mean	0.876	0.731	0.565	0.514	

L.S.D. Well water =0.068; Prolien =0.068; Interaction =0.136

The negative effect of the used irrigation water led to a significant decrease in the number of grains.  $\text{Pot}^{-1}$ . Where the results in Table (4) indicated that the use of high levels of salinity for irrigation water from 0 to 4.3 decimens/liter led

to a significant decrease in the characteristic rate by a decrease of 20.52%, and spraying with proline acid had a positive role in increasing the number of grains. When raising the concentration of proline acid from zero to 30 ppm, there was an increase in the characteristic rate with an increase of 13.18%. The difference was not significant between the concentrations of 20 and 30 ppm proline acid in the characteristic rate. As for the interaction between the two factors of the study, it was significant, and the highest value of the characteristic at concentration 0 for water type and concentration of 30 ppm proline acid was 176.00 compared with the lowest value at concentration 4.3 dS/L for water type and concentration 0 proline acid 125.50 with a decrease of 28.69%.

**Table 4. Effect of concentrations of irrigation water and spraying with proline acid and their interactions on the number of grains.**

Proline (ppm)	Well Water Concentration (ds/litter)				Mean
	0	1.6	3.2	4.3	
0	154.50	139.00	135.00	125.50	138.50
10	158.50	143.00	140.00	127.00	142.12
20	163.00	152.50	145.50	129.50	147.62
30	176.00	166.00	148.00	137.00	156.75
Mean	163.25	149.88	142.12	129.75	

L.S.D. Well water =5.99; Prolien =5.99; Interaction =11.98

As for the weight of 100 grains, it also decreased with an increase in the irrigation water concentration in the growth medium. The results in Table (5) indicated a significant decrease in the average weight of 100 grains with an increase in irrigation water levels from zero to 4.3 dS/L with a decrease of 34.24% and when the concentration of 100 grains was raised Proline acid from 0 to 30 ppm, there was a significant increase in the average weight of 100 grains, with an increase of 29.13 %. The difference was not significant between the concentrations 20 and 30 ppm of proline acid in the average characteristic. As for the interaction between the two factors of the study, it was significant, and the highest value for the trait at concentration 0 for water type and concentration of 30 ppm was 3.975 g, compared with the lowest value for the characteristic at concentration 4.3 dSi/liter for water type and concentration 0, proline acid, which amounted to 1.835 gm.

**Table 5. Effect of concentrations of irrigation water and spraying with proline acid and their interactions on the weight of 100 grains (gm) of wheat plant.**

Proline (ppm)	Well Water Concentration (ds/litter)				Mean
	0	1.6	3.2	4.3	
0	2.645	2.365	2.165	1.835	2.252
10	3.020	2.465	2.375	2.130	2.498
20	3.560	2.585	2.435	2.280	2.715
30	3.975	2.695	2.525	2.435	2.908
Mean	3.300	2.527	2.375	2.170	

L.S.D. Well water =0.223; Prolien =0.223; Interaction =0.447





The decrease in the yield of grains (gm. pot<sup>-1</sup>), the number of grains (pot<sup>-1</sup>) and the weight of 100 grains (gm) is due to the negative role of well water, as it affects the stage that begins from the detection and differentiation of spikelets to physiological maturity. So, it causes a reduction in the grain yield by reducing the number of fertile florets leading to a decrease in the yield and number of grains (Al-Saadi, 2010). Therefore, the negative impacts of well water can be represented in influencing the regulation of the distribution of nutrients represented between the source and the estuary.

In light of the results obtained from the previous tables, it can be noted the negative impact of well water on irrigation water and its increasing concentrations in all indicators studied in the experiment. Spraying plants with proline acid and its increasing concentrations had a positive role in reducing the negative impact of well water. There is no significant difference between the concentrations of 20 and 30 parts per million of proline acid in their effect on the studied indicators, and this is important from the economic side.

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## REFERENCES

- Al-Kateb, Y. M. 1988. Classification of Seed Plants. University of Baghdad, Ministry of Higher Education and Scientific Research, pp. 324-326.
- Al-Qazzaz, A. G. M. 2010. Effect of Spraying with Proline Acid on the Tolerance of Wheat Plant *Triticum Aestivum* L. Irrigated with Salt Water. Master's Thesis, Ibn Al-Haytham College of Education, University of Baghdad.
- Al-Saadi, A. J. H. H., A. Hassan, A. H. and A. G. M Al-Qazzaz. 2010. The Role of Proline Acid in Reducing the Negative Impact of Irrigation Water on Yield Components of Wheat. *Anbar Journal of Agricultural Sciences* 8:433-444.
- Ashraf, M. Athar, H.R.; P. J. C. Harris and T.R.Kwon . 2008. Some Prospective Strategies for Improving Crop Salt Tolerance. *Advances in agronomy* 97:45-110. [https://doi.org/10.1016/S0065-2113\(07\)00002-8](https://doi.org/10.1016/S0065-2113(07)00002-8)
- Bartels, D. and R. Sunkar. 2005. Drought and Salt Tolerance in Plants. *Critical. Review in Plant Sciences* 24:23-58.
- Ghassemi, F., A.J Jakeman, A.H. Nix .1995. Salinisation of land and water resources: human causes, extent, management and case studies. CAB international.
- Gratao, P.L. Polle, A. Lea, P.D. and R.A. Azevedo. 2005. Making the Life of Heavy Metal- Stressed Plant a little Easier. *Functional plant biology* 32:481-494. <https://doi.org/10.1071/FP05016>
- Hare, P.D. and W.A. Cress. 2004. Metabolic Implications of Stress-Induced Proline Accumulation in Plant. *Plant Growth Regulators* 21:79-102.
- Hussein, Z. K., and M. Q. Khursheed. 2014. Effect of foliar application of ascorbic acid on growth, yield components and some chemical constituents of wheat under water stress conditions. *Jordan Journal of Agricultural Sciences* 173:1-16.
- Ibrahim, H. A., and M.R Samiullah. 2010. Effect of exogenous application of osmolytes on growth and yield of wheat under drought conditions. *Journal of Environmental and Agricultural Sciences* 21:06-13.
- Little, T.M. and F.J.Hills. 1978. *Agricultural Experimentation Design and Analysis*, John Wiley and Sons, New York. U.S.A
- Munns, R., 2002. Comparative physiology of Salt and Water Stress. *Plant, cell & environment* 25:239-250.
- Parveen, A., M. Arslan Ashraf, Hussain, I., S. I Perveen, R. Rasheed, I. Mahmood, E.F. Abd Allah. 2021. Promotion of growth and physiological characteristics in water-stressed *Triticum aestivum* in relation to foliar application of salicylic acid. *Water* 13:1316.
- Rabie, G.H. and A.M. Almadini. 2005. Role of Bioinoculants in Development of Salt Tolerance of Vicia Faba Plants under Salinity Stress. *African Journal of Biotechnology* 4:210-222.
- Tan, J. Zhao, H. Hong, J. Han, H.Y. Li, and W. Zhao. 2008. Effects of Exogenous Nitric Oxide on Photosynthesis, Antioxidant Capacity and Proline Accumulation in Wheat Seedlings Subjected to Osmotic Stress. *World Journal of Agricultural Sciences* 4:307-313.
- Yassin, B. T. 1992. *Water Stress Physiology in Plants*. University of Mosul, Ministry of Higher Education and Scientific Research, Iraq. Pp. 134-137.
- Yassin, B. T. 2001. *Fundamentals of Plant Physiology*. College of Science, Qatar University, pp. 562-568.

